nomenon in both groups. For the first group of patients the support may ease their concern, for the second group preventive measures may be discussed.

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Thursday, February 26, 1998 9.00–18.00

Biology/Metabolism

P10 Multistep carcinogenesis of sporadic breast cancer

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Introduction: Breast cancer emerges as a multistep process with transformation of normal cells via steps of proliferation, atypia and in situ carcinoma. Cytogenetic and molecular genetic analysis of breast cancer samples indicate that tumor development involves the accumulation of various genetic alterations including amplification of oncogenes and mutation or loss of tumour suppressor genes. Microdissection of histological sections is needed to correlate the specific histological change and the genetic alteration. Up to date, no studies with prove of a direct sequential genetic motif are published, but the concomitant analyses of various genetic alterations in early lesions may correlate the histological finding with a biological function.

Methods: In a prospective study, after microdissection (1) DNA from 127 breast cancers with matched normal tissue was isolated and (2) in 20 cases comparative analyses with benign and precursor lesions (DCIS, CLIS) of the same breast were performed. Oncogene amplification (erbB2, int-2, c-myc, cdk4) was measured by quantitative differential PCR. Allele loss of tumor suppressor genes (p53, BRCA1, BRCA2, HIC1, MTS1/p16, NME1) was analysed by PCR-based microsatellite polymorphisms detecting differences in short tandem repeat sequences, which are informative for assessment of loss of heterozygosity (LOH). Fluorescent labelled PCR products were analysed and quantitated by polyacrylamid gel electrophoresis in an automated DNA sequencer (A.L.F.™Pharmacia, Freiburg, Germany). Results were analysed with Fragment Manager™ software.

Results: Prior to the study, DNA extraction from microdissected sections stained with different methods (hematoxylin, toluidin) as well as the linearity of the PCR reactions had to be validated. Oncogene amplification was found in 24% for erbB2, 19% for int2, 18% for c-myc, and 12% for cdk4. LOH could be detected in 57% for TP53, 48% for HIC1, in 38% for BRCA1, in 35% for BRCA2, in 8% for MTS1/p16. The sequential analyses shows LOH of TP53, LOH HIC1 followed by LOH of BRCA1 and c-myc amplification. In microdissected in situ lesions of the same breasts oncogene amplification and LOH could be demonstrated. These findings show identical alteration as seen in invasive samples.

Discussion: Quantitative differential PCR and microsatellite analyses combined with detection of fluorescent labelled PCR products by an automated laser DNA sequencer are powerful tools in determination of genetic alterations. Especially in combination with microdissection where small lesions are analysed, they proved to be useful as analytical methods. The accumulation and the combination of different genetic alterations may lead to a hint to the time frame of multistep carcinogenesis. The simultaneous analysis of histology and genetics of precursor lesions offers the opportunity for a biological description of the histological picture. The results of this pilot study support the concept of multistep carcinogenesis in breast cancer. LOH of TP53 in precursor and invasive lesions point to a key function of TP53 in breast cancer development.
(DFG Be 1215/6-2)

P11 Loss of retinoic acid receptor β expression in breast cancer and morphologically normal adjacent tissue but not in the normal breast tissue distant from the cancer

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Retinoids and their receptors (RARs, retinoic acid receptors; RXRs, retinoid X receptors) play an important role in maintaining the balance between proliferation and apoptosis. Recently, Deng and coworkers (*Science* 274: 2057–2059, 1996) reported a loss of heterozygosity on chromosome 3p24 in breast cancer specimens and the morphologically normal appearing adjacent tissue. The 3p24 locus includes, among other genes, the region coding for RAR-β. This study was designed to determine whether there are abnormalities in the expression of retinoid receptors in surgical specimens of patients with breast cancer.

In fourteen patients, transcripts of nuclear retinoid receptors were detected by *in situ* hybridization in formalin-fixed, paraffin-embedded specimens by means of digoxigenin-labeled riboprobes specific for RAR-α, -β and -γ.

We found RAR-α expressed in all specimens, whereas RAR-γ was expressed in 100% of normal breast tissue, but only in 11 out of 14 tumorous lesions. RAR-β was found in all cases of normal breast tissue localized distant from the tumor, but in 13 out of 14 cases it was completely absent in the tumor and the morphologically normal appearing tissue adjacent to the tumor. One possibility to explain the suppression of RAR-β is a mutation in the promoter region. Sequencing the DNA extracted from paraffin-embedded tumor tissue of the corresponding breast cancer specimens, we were not able to detect any mutation in the retinoic acid responsive element (RARE).

Our results clearly indicate a crucial role of RAR-β in the carcinogenesis of breast cancer.

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P12 In vitro cultivation of human mammary epithelial cancer cells. Study of their phenotypic characteristics and biologic behavior

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The successful long-term growth of tumor cells from primary breast tumor explants is a rare event. To define the characteristics of tumor cells which govern their ability to grow in vitro as primary culture as well as continuous or established cell lineage, human mammary epithelial cancer (HMEC) cells from 18 cases of unselected primary breast cancer were propagated in culture. Propagation of HMEC cells in vitro as monolayer in primary culture was successful in 10 out of 18 (55.5%) cases, which showed continuous proliferation of tumor cells only up to 6–8 passages before they reached senescence. An investigation of the effects of phenotypic expression of estrogen receptors (ER), the progesterone receptors (PgR), C-erb B_2 oncoprotein and epidermal growth factor receptors (EGFR) on the capacity of HMEC cells to grow in vitro as monolayers showed that expression of ER and EGFR is required for controlling tumor proliferative activity in vitro. Expression of ER made the growth of HMEC cells more difficult, while expression of EGFR protein made their growth in vitro easier. Phenotypic characteristics of floating HMEC cells were found to be different from those grown on coverslip as adherent cultures, suggesting a selective growth of HMEC cells of a specific phenotype in culture. This suggests that cell lines are not appropriate tool for chemosensitivity and radiosensitivity studies because neither the primary cells nor passaged cells represent the heterogeneous population of original tumor. Cultured HMEC cells in subsequent passages showed a decrease in their proliferative capacity, alterations in phenotypic characteristics and development of morphological features of terminal differentiation, resulting in senescence.

P13 Involvement of plasminogen activator inhibitor PAI-1 in *in vitro* growth of human breast cancer cell lines

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Introduction: Effective proteolysis of extracellular matrix is a critical factor for tumor growth and metastasis. The integrity of extracellular matrix is affected by the activity of several different classes of proteinases, like serine proteases such as plasmin, generated by the urokinase pathway of plasminogen activation. The later is regulated by a specific cell-surface uPA receptor (uPAR) and by two inhibitors (PAI-1, PAI-2). It has been proposed that high levels of PAI-1 may protect the tumor against degrading itself and in this way promoting tumor growth. We have addressed the question whether human breast cancer cell lines express in a different manner components of the plasminogen activation system and whether this expression is correlated with their *in vitro* growth rate.

Material and Methods: 1×10^4/ml cells of 2 estrogen receptor (ER) as well as progesterone receptor (PR) negative (BT-20, MX-1) and 1 ER, PR positive (MCF-7) human breast cancer cell lines were cultivated in DMEM/F12 serum-free medium up to 6 days. suPAR, uPA and PAI-1 immunoreactivity were assayed by Elisa. MTT-assay as described elsewhere (Mossmann, 1986) was applied to estimate the *in vitro* growth capacity.

Results: In all media, uPA- and uPAR immunoreactivity was detected. MCF-7 cells did not express any PAI-1, whereas BT-20 tumor cells secreted a 7 fold higher PAI-1 amount (0.57 ng/ml) than the MX-1 cell line (0.08 ng/ml). Comparable levels of urokinase plasminogen activator were measured by all three cell lines. Again, BT-20 cells exhibited higher levels of uPAR concentration than MX-1 or MCF-7 cells. Interestingly BT-20 and MCF-7 tumor cells had similar in